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Neurologin-2 and the tightrope of excitation/inhibition balance in the prefrontal cortex

Liang J, Xu W, Hsu Y-T, Yee AX, Chen L, Südhof TC. Conditional neurologin-2 knockout in adult medial prefrontal cortex links chronic changes in synaptic inhibition to cognitive impairments. *Mol Psychiatry* 20: 850-859, 2015.

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ABSTRACT

Excitation/inhibition imbalance is implicated in symptoms of neuropsychiatric disorders. We discuss a study by Liang et al. (2015) demonstrating that the conditional knockout of neuroligin-2, a postsynaptic adhesion protein, in the prefrontal cortex of adult mice results in alterations in inhibitory synaptic properties. However, behavioral impairments emerged prior to the development of detectable changes in excitation/inhibition ratio. This suggests there may be network-specific excitation/inhibition ratios, some of which are more vulnerable to disruption than others.

The brain is a delicate and extraordinarily complex structure whose proper wiring underlies all our behaviors, thoughts, emotions, memories and interactions with the external world. Healthy brains walk the tightrope between excitation and inhibition, and this balance is essential for nearly all functions, as it helps to maintain the constancy of the internal environment. The equilibrium between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission is established during development and maintained throughout life to prevent neural circuits from becoming hyper- or hypoactive. The breakdown of this delicate balance has been hypothesized to underlie neuropsychiatric disorders such as autism and schizophrenia. This hypothesis proposes that the diverse genetic factors involved in these disorders result in similar psychiatric symptoms by converging to a common neural circuit pathology. It is supported by evidence from human studies that molecular changes such as anomalous expression of proteins involved in GABA biosynthesis and neurotransmission in the prefrontal cortex (PFC) might perturb the proper balance between excitation and inhibition (Coghlan et al., 2012).

It has recently been shown that the acute optogenetic elevation of excitation/inhibition (E/I) balance in the medial PFC (mPFC) causes behavioral impairments akin to those observed in

neuropsychiatric patients (Yizhar et al., 2011), consistent with the well-documented role of the PFC in cognition, sociability, and other executive functions. However, although very informative, studies using acute manipulations provide only limited insights into how the brain reacts to chronic changes in neural activity that form the basis of most neuropsychiatric disorders. At the other extreme, the traditional techniques of targeted gene manipulation are systemic in nature (e.g., lacking spatial and/or temporal selectivity) and often induce adaptive and compensatory mechanisms. Thus, direct evidence for the hypothesis that chronic dysregulation of E/I balance in the PFC causes impairments associated with neuropsychiatric disorders is still lacking.

Important support for his hypothesis was recently provided by the study by Liang et al. (2015), who identified neuroligin-2 (Nlgn2) as a link between chronic changes in the E/I balance in the mPFC and behavioral impairments resembling those seen in neuropsychiatric patients. Neuroligins are ubiquitously expressed postsynaptic adhesion proteins that play a key role in the regulation of synapse organization and function (Sudhof, 2008). Of the four neuroligin isoforms described in rodents, Nlgn1 and Nlgn2, that are found at excitatory and inhibitory synapses respectively, have been studied most extensively (Sudhof, 2008). Their selective localization at particular types of synapses makes them uniquely suited for controlling the balance between excitation and inhibition. Indeed, the knockout (KO) of *Nlgn1* was reported to impair NMDA-receptor mediated glutamatergic signaling (Sudhof, 2008), whereas manipulations of Nlgn2 levels were shown to result in altered inhibitory synaptic transmission (Sudhof, 2008). In humans, mutations in neuroligin genes have been linked to autism and schizophrenia, suggesting that they are critical for normal brain function. Moreover, mice deficient in these proteins exhibit impaired synaptic function, learning and memory, and abnormal social behaviors (Wohr et al., 2013).

Liang et al. (2015) achieved control of synaptic properties in a chronic, locally restricted and specific manner by creating conditional knockout (cKO) mice of *Nlgn2*, a multipurpose tool allowing the assessment of 1) *Nlgn2* function in the mPFC, 2) manipulation of mPFC activity levels and examination of its functional outcomes, and 3) evaluation of endophenotypes associated with neuropsychiatric disorders. *Nlgn2* protein was already undetectable in the mPFC 2-3 weeks after administration of Cre-recombinase to young adult mice. However, a decrease in the density of inhibitory, GABAergic synaptic terminals only took place 6-7 weeks post-manipulation. This gradual loss of inhibitory synapses suggested a possible reduction of inhibitory synaptic transmission, a finding previously observed in conventional *Nlgn2* KO mice (Sudhof, 2008). Indeed, Liang et al. (2015) found that whole-cell patch clamp recordings in layer 2/3 pyramidal neurons revealed impoverished inhibitory neurotransmission 6-7 weeks after the mPFC-specific deletion of *Nlgn2*. This slowly progressing reduction of inhibitory neurotransmission suggests that *Nlgn2* is important for long-term maintenance and restructuring of synapses, i.e., for synaptic plasticity. The ability of neurons to remodel their network of connections underlies most complex adaptive behaviors, and consequently, deficits in synaptic plasticity in the mPFC have been implicated in various neuropsychiatric disorders (Goto et al., 2010). Interestingly, the effect of chronic *Nlgn2* deletion on synaptic transmission was stronger than its effect on inhibitory synapse numbers, suggesting the involvement of additional mechanisms besides the loss of inhibitory synapses. It is of note, that a reduction in synapse numbers has not been observed in full *Nlgn2* KO mice (Blundell et al., 2009) despite a decrease in the density of VGAT-positive puncta, thereby calling for closer, electron microscopy examination of synapse numbers in *Nlgn2* cKO mice. Furthermore, the analysis of evoked synaptic responses uncovered a reduction of inhibitory postsynaptic currents amplitude, but not of their excitatory counterpart, leading to increased E/I ratio 6-7 weeks after *Nlgn2* deletion in the mPFC. Consistent with a

previous report of preferential requirement of *Nlgn2* in perisomatic synapses originating from fast spiking, i.e., parvalbumin-expressing, interneurons (Gibson et al., 2009), selective ablation of *Nlgn2* in the mPFC also seemed to selectively impair inhibitory synapses on the soma and/or on the basal dendrites. This finding is well aligned with a recent report of Xue et al. (2014) that parvalbumin-, but not somatostatin-expressing inhibitory neurons participate in maintenance of E/I equilibrium. Moreover, dysfunction of parvalbumin-expressing interneurons has been associated with several neuropsychiatric disorders (Hu et al., 2014), thus linking the findings of Liang et al. (2015) with E/I imbalance in mPFC observed in patients with autism and schizophrenia (Coghlan et al., 2012).

Prompted by these electrophysiological findings, Liang et al. (2015) investigated whether this *Nlgn2* ablation-induced reduction in synaptic inhibition in the mPFC impacted the behavior of *Nlgn2* cKO mice. Whereas locomotor activity and spatial memory were not affected, *Nlgn2* cKO animals exhibited reduced anxiety in the elevated plus maze test, both 2-3 weeks and 6-7 weeks after the manipulation. This finding is quite surprising as reduction in inhibition is intuitively expected to result in elevated anxiety, as observed in the full *Nlgn2* KO mice (Blundell et al., 2009; Wöhr et al., 2013). This discrepancy in results related to anxiety might reflect differences in conventional vs. conditional knockout strategies. Combined with the finding that the commonly used open field test did not show reduced anxiety in the Liang et al. (2015)'s study, this calls for further investigation of this behavioral phenotype in *Nlgn2* cKO mice. In addition to lower anxiety level, Liang et al. (2015) documented decreased contextual and cued fear conditioning in *Nlgn2* cKO mice, both 2-3 and 6-7 weeks after *Nlgn2* inactivation. Interestingly, no impairments in fear memory were observed when fear-conditioning assays were conducted on mice that were trained prior to *Nlgn2* deletion, implying that mPFC-specific *Nlgn2* inactivation primarily affects memory consolidation, but not its retrieval. Moreover, this elegant experiment also excluded reduced

anxiety as a possible confounding factor contributing to the impairment in fear conditioning. Finally, these memory deficits seemed to be specific to mPFC-dependent memories as *Nlgn2* cKO mice exhibited normal motor learning ability in a rotarod task. As rotarod deficits were seen in the full *Nlgn2* KO mice (Blundell et al., 2009; Wohr et al., 2013), this reiterates differences between conditional and conventional gene expression strategies and the importance of the former for elucidation of endophenotypes of complex neuropsychiatric disorders.

Alterations in anxiety, often seen in neuropsychiatric patients, are usually associated with impairments in social interactions. Surprisingly, however, instead of the increased sociability that might be expected from reduced anxiety, *Nlgn2* cKO mice exhibited decreased social interactions in a three-chamber test, but only 6-7 weeks after *Nlgn2* deletion. This finding stands in stark contrast with previously reported normal sociability in full *Nlgn2* KO mice (Blundell et al., 2009; Wohr et al., 2013). Thereby, it restates the importance of conditional gene manipulations, as the emergence of adaptive mechanisms in conventional KO animal models might compensate for a lost gene and thus hinder its functional role.

In addition to electrophysiological and behavioral assessments, Liang et al. (2015) performed immunohistochemical examinations of the ability of the *Nlgn2*-ablated mPFC to respond to salient experiences, such as fear conditioning and social interactions with conspecifics. By measuring the expression levels of c-Fos as a proxy for neural activity, following the two aforementioned behavioral tests, Liang et al. (2015) observed a pronounced decrease in mPFC c-Fos response, indicating reduced responsivity of mPFC to external inputs. Although this is seemingly counterintuitive given the observed mPFC disinhibition, this finding is consistent with the previous observations that the most robust activation of immediate-early genes is obtained only after lowering the background activity of neurons before applying a specific stimulus. Therefore, it seems plausible that reduced inhibition in the mPFC of *Nlgn2*

cKO mice may lead to an increase in the background “noise” caused by activity of mPFC neurons, which “desensitizes” these neurons and ultimately reduces their responsivity to external stimuli. Although resembling the observation of reduced c-Fos activation in a mouse model of fragile X syndrome (Krueger et al., 2011), this finding stands in contrast to a recent report of increased c-Fos activation following acute elevation of E/I ratio in the mPFC (Yizhar et al., 2011). As neuropsychiatric disorders usually arise from chronic rather than acute E/I impairments, this finding reiterates the value and need for studies examining such chronic, post-developmental changes.

Although the findings of Liang et al. (2015) suggest a link between behavioral impairments and alterations in the mPFC E/I ratio, it is perplexing that the behavioral impairments observed in *Nlgn2* cKO mice (with the exception of social deficits) preceded electrophysiological abnormalities: the behavioral phenotype was nearly as severe at the assessment 2-3 weeks post-manipulation as it was at 6-7 week after *Nlgn2* inactivation, whereas electrophysiological impairments were much stronger at the second time point. This is a particularly important finding, as it is often assumed that neurophysiological changes provide a substrate for and therefore precede the behavioral impairments in genetic models of neuropsychiatric disorders. To account for this discrepancy, Liang et al. (2015) suggest that the behavioral phenotype might stem from the dysfunction of a subpopulation of highly plastic and more actively remodeled inhibitory synapses, that are too few in numbers to affect the global electrophysiological recordings of inhibitory transmission in the mPFC. It is easy to imagine that the loss of *Nlgn2* could impair such neuron populations rapidly by preventing the plasticity needed for adaptive behaviors, while other subtypes of mPFC neurons with a more stable network of connections would be less vulnerable to the *Nlgn2* deletion. It is also plausible that different subtypes of mPFC neurons, which differentially project to subcortical structures, exhibit distinct sensitivity to a reduction in local inhibition. An additional,

intriguing possibility, proposed by van de Lagemaat et al. (2014), is that altered E/I ratio is not a cause but rather a consequence of behavioral symptoms. In that respect, it would be highly interesting to see if restoring the E/I balance by decreasing excitatory transmission in the mPFC of *Nlgn2* cKO mice would improve or normalize their behavioral phenotype.

The lack of correlation between the effects of *Nlgn2* deletion on synapses, behavior and neural activity raises the question of whether the E/I ratio, as a measure of the general state of a neural network, can capture the complexity of the neural correlates of behavior. While abnormalities in E/I ratio have been put forward as a possible biomarker of neuropsychiatric disorders, the deficits in neurotransmission underlying behavioral symptoms are likely more complex. This is an important finding with many potentially far reaching consequences. A lot of research resources have recently been put into understanding the causes of E/I imbalance in neuropsychiatric disorders and developing new treatments that aim to normalize the E/I ratio in the hope of improving disease symptoms. Furthermore, in the human neuroimaging literature as well as in rodent models research, there is much interest on the use of magnetic resonance spectroscopy as an *in vivo* measure of E/I balance. By showing that behavioral impairments can develop in the absence of detectable changes in E/I ratio, Liang et al. (2015)'s study raises the possibility that different neural networks within the same tissue might each have their own E/I ratio. In other words there may be not one tightrope but several, some of which more easily perturbed than others.

In summary, by using a comprehensive set of tools, Liang et al. (2015) have unraveled the post-developmental roles of *Nlgn2* in the mPFC and showed that its loss causes elevation of the E/I ratio and accompanying behavioral impairments. With this, they introduced a further level of complexity to our current understanding of the neurobiological underpinnings of neuropsychiatric disorders. In addition to providing direct evidence that dysfunctional activity in the adult mPFC is sufficient to cause behavioral deficits reminiscent of those seen

in neuropsychiatric patients, Liang et al. (2015)'s findings represent an important step towards the development of new treatments targeting the causes of neuropsychiatric disorders, and not just their symptoms. Although further research is needed to clarify the causality between changes in E/I balance and behavioral impairments, and to elucidate the potential translatability of these findings to clinical settings, this study by Liang et al. (2015) holds promise to open up a new avenue in research and modelling of neuropsychiatric disorders.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.M.P. conception and design of research; A.M.S.D., J.H. and M.M.P. drafted manuscript; A.M.S.D., J.H. and M.M.P. edited and revised manuscript; A.M.S.D., J.H. and M.M.P. approved final version of manuscript.